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2008 AFS Symposium Summary

Summary of the 2008 AFS Symposium: From Gels to Genomics: The Evolving Landscape of Pteridology. A Celebration of Gerald Gastony's Contributions to Fern Evolutionary Biology.—The study of pteridophyte evolutionary biology has undergone remarkable developments during the past 40 years. Central to these developments have been the efforts of Gerald J. Gastony and his academic offspring to advance our understanding of these plants. Accordingly, on 29 July, 2008, during the Botany 2008 Conference in Vancouver, British Columbia, former students, colleagues, and friends gathered to celebrate Jerry Gastony's productive career at the forefront of pteridology. The symposium highlighted some of the major methodological and philosophical advances that have evolved during his exemplary career. Trained as a classical taxonomist, Prof. Gastony has continually reinvented himself since arriving at Indiana University in 1970. His initial forays into enzyme electrophoresis shed light on such diverse topics as the breeding system of ferns, the role of cryptic taxa in reticulate lineages, and the contributions of paleo- and neopolyploidy to fern systematics and evolution. These questions have been persistent throughout Jerry's career and have been influential in shaping the field of pteridophyte evolutionary biology. The 2008 AFS symposium revisited these questions and showed how new tools are building on the foundation that Prof. Gastony helped lay over the last 40 years. In lieu of a formal Proceedings, the present text presents a brief summary of each of the presentations from the symposium, credited individually to each speaker and his co-authors.—Edited by Michael S. Barker, Department of Botany, University of British Columbia, 3529-6270 University Blvd, Vancouver, BC V6T 1Z4, CANADA, and Department of Biology, Indiana University Jordan Hall 142, 1001 E Third St., Bloomington, IN 46405-3700 and George Yatskievych, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

A Brief History of Gerald J. Gastony's Botanical Career.—After graduating from St. Ignatius High School in Cleveland, Ohio, Gerald J. Gastony (1940–) attended St. Louis University for his undergraduate training. His initial focus was on the humanities and in 1964 he received his Bachelor's Degree in the College of Philosophy and Letters. Through this focus, he became fluent in Latin and comfortable in Greek, skills that aided his future career as a plant systematist. Jerry also became interested in botany through a course from the distinguished taxonomist and floristician, John Dwyer, and he wound up taking the equivalent of a major's worth of classes in biology and supporting sciences in addition to those in his major. Dwyer subsequently encouraged Jerry to apply to Tulane University, where eventually he was advised by the noted naturalist and botanical historian, Joseph Ewan while supported by a predoctoral fellowship from NASA. It was during his work at Tulane that Jerry

became interested in ferns, which would be the focus of his doctoral work and future career. Ewan and Walter Hodge (then at NSF) were among those who encouraged Jerry to accept a Master's Degree (in 1966) from Tulane and to apply to the doctoral program at Harvard University (although this meant abandoning his NASA fellowship for support through a grant from NSF). There, he completed his Ph.D. in 1971 under Rolla Tryon, one of the preeminent classical fern systematists of his time.

Jerry's doctoral work on the taxonomy of the tree fern genus *Nephelea* (Gastony, 1973) not only prepared him for a career in systematics, but it also stimulated his interest in related topics, such as the comparative morphology of fern spores, variation in the fern life cycle, and speciation. Jerry accepted a faculty position at Indiana University in 1970, straight from graduate school. His initial research in Bloomington focused primarily on the spore morphology of tree ferns (Gastony, 1974, 1979, 1981, 1982; Gastony and Tryon, 1976).

However, several years into his position, Jerry became aware that in order to lead a successful career in a department that emphasized evolutionary studies beyond the organismal level, he would have to expand the focus of his research to address basic questions in evolutionary biology. In order to gain technical skills that would allow him to broaden his research program. Jerry sat in on several courses at Indiana University on biochemistry and genetics. He then applied this knowledge to a new effort to adapt the developing field of isozyme electrophoresis to ferns. He also spent his first sabbatical in Leslie Gottlieb's lab at the University of California at Davis, where he perfected his isozyme techniques and began to apply them to evolutionary and population genetic studies in ferns. At the time, existing protocols to extract, resolve, and genetically interpret the banding patterns of common enzyme systems mostly did not work with ferns (Soltis *et al.*, 1983), and Jerry was challenged to prove himself in the Gottlieb lab. Ferns in the genus *Pellaea* are abundant and cytologically diverse in California, and these became Jerry's model system for many future studies involving taxonomic relationships, population genetics, formation of polyploids, and the contributions of apogamous taxa to fern evolution (Gastony and Gottlieb, 1982, 1985; Gastony, 1988, 1990, 1991, Gastony and Windham, 1989).

The coupling of classical and molecular techniques led to Jerry's pioneering work on fern isozymes, and his lab (known as "Sky Lab" because of its location on the top floor of Jordan Hall) became a popular destination and invaluable resource for graduate and postdoctoral students interested in plant systematics and evolution. In the mid-1980s, Jerry and his students and collaborators further expanded the lab's repertoire to include restriction-site variation of DNA. Jerry's lab was one of the first to use variation in fern chloroplast DNA to understand historical relationships among fern species and genera (Yatskievych *et al.*, 1988, Stein *et al.*, 1989; Gastony *et al.*, 1992). A few years later, Jerry began studying DNA sequence data for phylogenetic analyses of ferns, which eventually led to the first comprehensive phylogeny for ferns (Hasebe *et al.*, 1995). Most recently, his lab generated the first genetic linkage map for a

FIG. 1. Gerald J. Gastony working in the greenhouse at Indiana University in 2008.

fern, which will provide an important and permanent resource for fern genetics (Nakazato *et al.*, 2006).

Because of the great diversity of Jerry's contributions to fern systematics and evolution, it is difficult to summarize all of them here. For example, his early work on spore morphology of the Cyatheaceae (Gastony, 1974, 1979; Gastony and Tryon, 1976) provided some of the initial evidence that the prevailing generic classification was unnatural. He was the first to count the chromosomes of the sporophyte-less taxon, *Vittaria appalachiana* Farrar & Mickel, which required adapting existing cytological protocols to the special demands of mitotic cells in gametophytic tissue (Gastony, 1977). He also demonstrated that ferns have diploid isozyme expression patterns despite their high chromosome numbers and that, contrary to prevailing wisdom at the time, homosporous ferns are highly heterozygous rather than homozygous (Gastony and Gottlieb, 1982, 1985). He later showed that fern genes can become silenced following genome doubling (Gastony, 1991). His work on cheilanthoid ferns provided the first robust phylogeny of that large and taxonomically difficult group (Gastony and Rollo, 1995, 1998), but he also has made substantial contributions to the understanding of other fern groups, in such families as Apleniaceae (Gastony, 1971; Gastony, 1986; Gastony and Johnson, 2001), Onocleaceae (Gastony and Ungerer, 1997), and other subfamilies of Pteridaceae (Gastony and Baroutsis, 1975; Baroutsis and Gastony, 1978; Gastony and Johnson, 2001; Nakazato and Gastony, 2003).

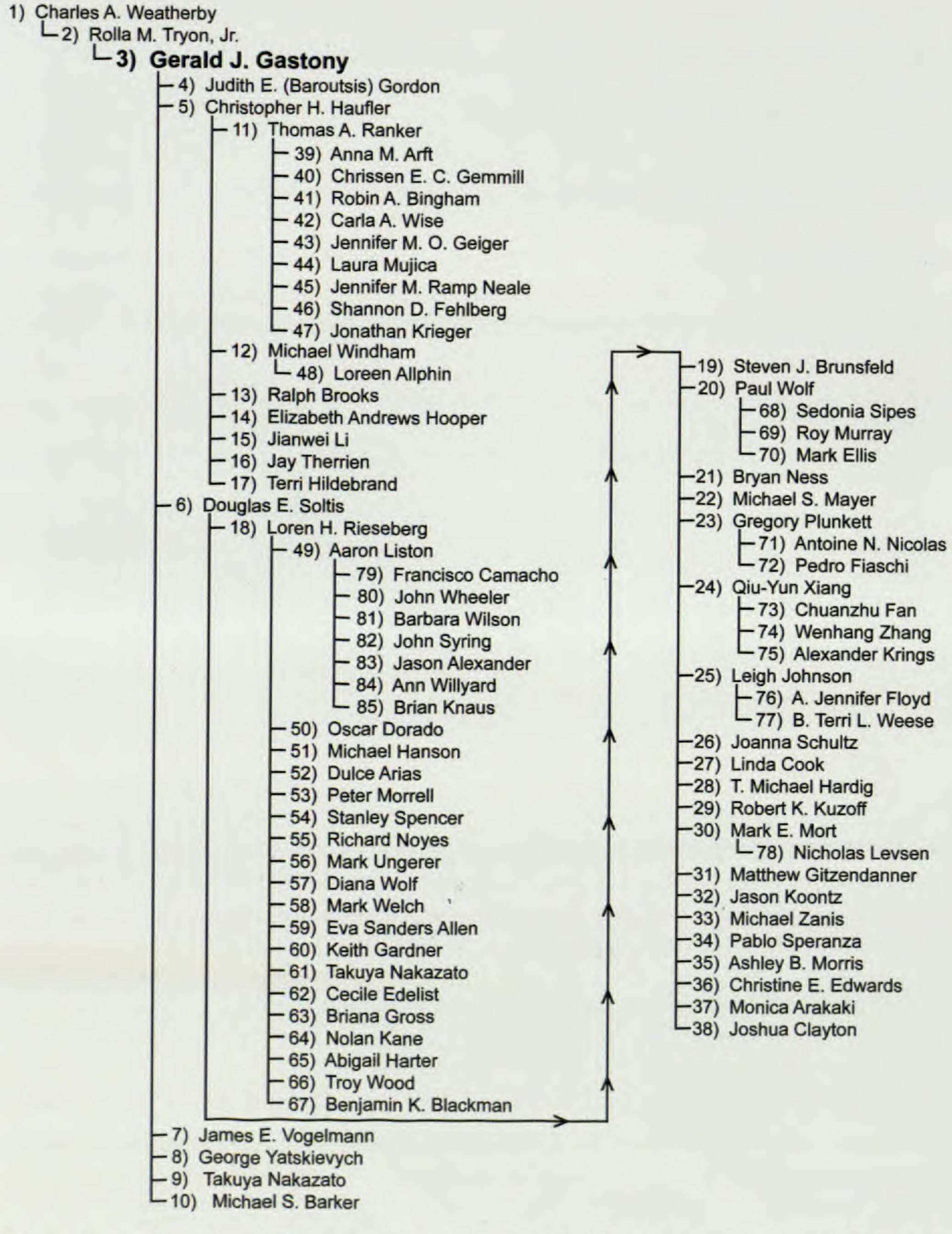

FIG. 2. An academic genealogy of Gerald J. Gastony, his botanical lineage, and his academic descendants. See Table 1 for further information on each person named.

In 1995, Jerry Gastony (Fig. 1) received the Edgar T. Wherry Award from the Botanical Society of America (Anonymous, 1995). In 2006, he was one of the honorees for a Centennial Medallion Award from the Botanical Society of America. He was chairman of the Pteridological Section of the Botanical

TABLE 1. Biographical summary of individuals in the Academic Genealogy of Gerald J. Gastony. See Fig. 2 for chronology and context.

1. Charles A. Weatherby. Academic grandfather. See *American Fern Journal* 40(1) for information.
2. Rolla M. Tryon, Jr. Academic father. Ph.D. Harvard University, 1941. See *American Fern Journal* 92(1): 1–9, 2002 for further information.
3. Gerald J. Gastony. Ph.D. 1971, Harvard University. Currently Professor of Biology Emeritus, Indiana University, Bloomington.
4. Judith E. (Baroutsis) Gordon. Ph.D. 1976 (as Judith G. Baroutsis), Indiana University, Bloomington. Currently Professor of Biology Emerita, Department of Biology, Augusta Sate University, Augusta, GA.
5. Christopher H. Haufler. Ph.D. 1977, Indiana University, Bloomington. Currently Professor and Chair, Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence.
6. Douglas E. Soltis. Ph.D. 1980, Indiana University, Bloomington. Currently Professor and Chair, Department of Botany, University of Florida, Gainesville.
7. James E. Vogelmann. Ph.D. 1983, Indiana University, Bloomington. Currently Research Ecologist, U.S. Geological Survey Earth Resources Observation and Science Center, Sioux Falls, SD and Adjunct Professor, South Dakota State University, Brookings.
8. George Yatskievych. Ph.D. 1990, Indiana University, Bloomington. Currently Curator and Director of the Flora of Missouri Project, Missouri Botanical Garden, St. Louis and Research Associate Professor and Adjunct Graduate Faculty, University of Missouri–St. Louis and Research Associate, Arizona-Sonora Desert Museum, Tucson.
9. Takuya Nakazato. Ph.D. 2005, Indiana University, Bloomington. Co-advised by Loren H. Rieseberg. Currently Assistant Professor, Department of Biology, University of Memphis, Memphis, Tennessee. Also see number 60,
10. Michael S. Barker. Ph.D. 2009, Indiana University, Bloomington. Co-advised by Loren H. Rieseberg. Currently Postdoctoral Associate, Department of Botany, University of British Columbia, Vancouver.
11. Thomas A. Ranker. Ph.D. 1987, University of Kansas. Currently Professor and Chair, Department of Botany, University of Hawaii at Manoa, Honolulu, HI.
12. Michael Windham. Ph.D. 1988, University of Kansas. Currently Research Scientist and Curator of Vascular Plants, Department of Biology, Duke University, Durham, NC.
13. Ralph Brooks. Ph.D. 1989, University of Kansas. Currently Senior Environmental Scientist Black & Veatch, Lake Oswego, OR.
14. Elizabeth Andrews Hooper. Ph.D. 1994, University of Kansas. Currently Associate Professor of Biology, Truman State University, Kirksville, MO.
15. Jianwei Li. Ph.D. 1996, University of Kansas. Currently Bioinformatics Engineer III, J. Craig Venter Institute, Rockville, MD.
16. Jay Therrien. Ph.D. 2003, University of Kansas. Currently Director of Sales, Asia Pacific and Japan, Illumina, Inc., Scoresby VIC, Australia.
17. Terri Hildebrand. Ph.D. 2005, University of Kansas. Currently Assistant Professor of Botany, Department of Biology, Southern Utah University, Cedar City, UT.
18. Loren H. Rieseberg. Ph.D. 1987, Washington State University. Currently Professor and Canada Research Chair, Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada and Distinguished Professor, Department of Biology, Indiana University, Bloomington.
19. Steven J. Brunsfeld. Ph.D. 1990, Washington State University. Professor, Department of Forest Resources, University of Idaho, Moscow. Deceased, 2007 (http://www.cnrhome.uidaho.edu/default.aspx?pid=96887).
20. Paul Wolf. Ph.D. 1990, Washington State University. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Professor, Department of Biology, Utah State University, Logan.
21. Bryan Ness. Ph.D. 1992, Washington State University. Currently Associate Professor, Department of Biology, Pacific Union College, Angwin, CA.

TABLE 1. Continued.

22. Michael S. Mayer. Ph.D. 1993, Washington State University. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Associate Professor, Department of Biology, University of San Diego, San Diego, CA.
23. Gregory Plunkett. Ph.D. 1994, Washington State University. Currently Curator and Director, Cullman Program in Molecular Systematic Studies, The New York Botanical Garden, Bronx, NY.
24. Qiu-Yun Xiang. Ph.D. 1995, Washington State University. Currently Associate Professor, Department of Plant Biology, North Carolina State University, Raleigh, NC.
25. Leigh Johnson. Ph.D. 1996, Washington State University. Currently Associate Professor and Herbarium Curator, Department of Biology, Brigham Young University, Provo, UT.
26. Joanna Schultz. Ph.D. 1996, Washington State University. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Senior Consultant, Earth Informations Systems, Houston, TX.
27. Linda Cook. Ph.D. 1998, Washington State University. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Lecturer part time, Washington State University, Pullman.
28. T. Michael Hardig. Ph.D. 1998, Washington State University. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Associate Professor, Department of Biology, Chemistry, and Mathematics, University of Montevallo, Montevallo, AL.
29. Robert K. Kuzoff. Ph.D. 1998, Washington State University. Co-advised by Larry Hufford. Currently Associate Professor, Department of Biological Sciences, University of Wisconsin, Whitewater.
30. Mark E. Mort. Ph.D. 1999, Washington State University. Currently Associate Professor, Department of Ecology and Evolutionary Biology and Associate Curator of the McGregor Herbarium, University of Kansas, Lawrence.
31. Matthew Gitzendanner. Ph.D. 2000, Washington State University. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Associate Scientist, Department of Botany, University of Florida, Gainesville.
32. Jason Koontz. Ph.D. 2000, Washington State University. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Assistant Professor, Department of Biology, Augustana College, Rock Island, IL.
33. Michael Zanis. Ph.D. 2002, Washington State University. Currently Assistant Professor, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN.
34. Pablo Speranza. Ph.D. 2005, University of Florida. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Profesor Adjunto de Fitotecnia, Departamento de Biología Vegetal, Universidad de la República, Montevideo, Uruguay.
35. Ashley B. Morris. Ph.D. 2006, University of Florida. Advised by Pamela Soltis, co-advised by Douglas Soltis. Currently Assistant Professor, Department of Biology, University of South Alabama, Mobile.
36. Christine E. Edwards. Ph.D. 2007, University of Florida. Co-advised by Douglas Soltis and Pamela Soltis. Currently Postdoctoral Research Scientist, Department of Botany, University of Wyoming, Laramie.
37. Monica Arakaki. Ph.D. 2008, University of Florida. Currently Postdoctoral Fellow, Department of Ecology and Evolutionary Biology, Brown University, Providence, RI.
38. Joshua Clayton. Ph.D. 2008, University of Florida. Currently seeking employment in UK.
39. Anna M. Arft. Ph.D. 1995, University of Colorado, Boulder. Currently homemaker.
40. Chrissen E. C. Gemmill. Ph.D. 1996, University of Colorado, Boulder. Currently Senior Lecturer (≈ Associate Professor in U.S.), Department of Biological Sciences, University of Waikato, Aotearoa, New Zealand.
41. Robin A. Bingham. Ph.D. 1997, University of Colorado, Boulder. Currently Professor, Department of Natural and Environmental Sciences, Western State College, Gunnison, CO.

TABLE 1. Continued.

42. Carla A. Wise. Ph.D. 1997, University of Colorado, Boulder. Yan Linhart, co-advisor. Currently independent Environmental and Science Writer, Corvallis, OR.
43. Jennifer M. O. Geiger. Ph.D. 2003, University of Colorado, Boulder. Currently Associate Professor, Department of Natural Sciences, Carroll College, Helena, MO.
44. Laura Mujica. Ph.D. 2004, University of Colorado, Boulder (as Laura Mujica-Crapanzano). Patrick Bourgeron co-advisor. Currently Term Assistant Professor, Chemistry Department, University of Alaska, Anchorage, AK.
45. Jennifer M. Ramp Neale. Ph.D. 2005, University of Colorado, Boulder. Sharon Collinge, co-advisor. Currently Associate Director of Research and Director of the Conservation Genetics Program, Denver Botanic Gardens, Denver, CO.
46. Shannon D. Fehlberg. Ph.D. 2006, University of Colorado, Boulder. Currently Conservation Biologist, Desert Botanical Garden, Phoenix, AZ.
47. Jonathan Krieger. Ph.D. 2007, University of Colorado, Boulder. Robert P. Guralnick co advisor. Currently Postdoctoral Research Associate, Department of Palaeontology, The Natural History Museum, London.
48. Loreen Allphin. Ph.D. 1996, University of Utah, Salt Lake City. Advised by Delbert Wiens, co-advised by Michael Windham. Currently Associate Professor, Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT.
49. Aaron Liston. Ph.D. 1990, Claremont Graduate University, Claremont, CA. Nominally advised by Thomas S. Elias, co-advised by Loren H. Rieseberg. Currently Professor, Department of Botany and Plant Pathology and Director of the OSU Herbarium, Oregon State University, Corvallis, OR.
50. Oscar Dorado. Ph.D. 1992, Claremont Graduate University. Currently Professor, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico.
51. Michael Hanson. Ph.D. 1993, Claremont Graduate University. Currently tenured botany Instructor, Bellevue College, Bellevue, WA.
52. Dulce M. Arias. Ph.D. 1994, Claremont Graduate University. Currently Professor, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico.
53. Peter Morrell. Ph.D. 1997, Claremont Graduate University. Currently Senior Research Geneticist, Monsanto Co., St Louis, MO.
54. Stanley Spencer. Ph.D. 1997, Claremont Graduate University. Currently Senior Biologist at LSA Associates, Inc. [environmental consulting firm], Riverside, CA.
55. Richard Noyes. Ph.D. 1999, Indiana University, Bloomington. Currently Assistant Professor, Department of Biology, University of Central Arkansas, Conway, AR.
56. Mark Ungerer. Ph.D. 2000, Indiana University, Bloomington. Currently Assistant Professor, Division of Biology, Kansas State University, Manhattan, KS.
57. Diana Wolf. Ph.D. 2000, Indiana University, Bloomington. Currently Assistant Professor, Institute of Arctic Biology, University of Alaska, Fairbanks AK.
58. Mark Welch. Ph.D. 2002, Indiana University, Bloomington. Currently Assistant Professor, Department of Biological Sciences, Mississippi State University, Mississippi State, MS.
59. Eva Sanders Allen. Ph.D. 2002, Indiana University, Bloomington. Ellen Ketterson, co-advisor. Currently Grants Specialist, Department of Biology, Indiana University, Bloomington.
60. Keith Gardner. Ph.D. 2004, Indiana University, Bloomington. Currently Postdoctoral Fellow, Royal Botanic Garden, Edinburgh, UK.
61. Takuya Nakazato. Ph.D. 2005, Indiana University, Bloomington. Co-advised by Gerald J. Gastony. Currently Assistant Professor, Department of Biology, University of Memphis, Memphis, Tennessee. Also see number 9.
62. Cécile Edelist. Ph.D. 2007, Université Paris-Sud 11, Orsay, France. Advised by Christine Dillmann and Delphine Sicard, co-advised by Loren H. Rieseberg. Currently research engineer, Conservation des Espèces, Restauration et Suivi des Populations, National Museum of Natural History, Paris, France.

TABLE 1. Continued.

63. Briana Gross. Ph.D. 2007, Indiana University, Bloomington. Co-advised by Elizabeth Kellogg, University of Missouri, St. Louis. Currently Postdoctoral Research Fellow, Department of Biology, Washington University, St. Louis, MO.
64. Nolan Kane. Ph.D. 2007, Indiana University, Bloomington. Currently Postdoctoral Research Associate, Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada.
65. Abigail Harter. Ph.D. 2008, Indiana University, Bloomington. Currently Postdoctoral Fellow, University of Edinburgh, UK.
66. Troy Wood. Ph.D. April 2009, Indiana University, Bloomington.
67. Benjamin K. Blackman. Ph.D. May 2009, Indiana University, Bloomington. Co-advised by Scott Michaels.
68. Sedonia Sipes. Ph.D. 2001, Utah State University. Currently Associate Professor, Department of Plant Biology, Southern Illinois University, Carbondale, IL.
69. Roy Murray. Ph.D. 1997, Utah State University. Currently code hacker, IEM.
70. Mark W. Ellis. Ph.D. May, 2009, Utah State University, Logan, UT.
71. Antoine N. Nicolas, Ph.D. May 2009, Virginia Commonwealth University, Richmond, VA.
72. Pedro Fiaschi Ph.D. anticipated, August 2009, Virginia Commonwealth University, Richmond, VA.
73. Chuanzhu Fan. Ph.D. 2003, North Carolina State University. Currently Assistant Research Scientist, Arizona Genomics Institute, University of Arizona, Tucson, AZ
74. Wenhang Zhang. Ph.D. 2006, North Carolina State University. Michael Purugganan, co-advisor. Currently Postdoctoral Fellow, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.
75. Alexander Krings. Ph.D. 2007, North Carolina State University. Jon M. Stucky, co-advisor. Currently Extension Assistant Professor and Director of the Herbarium, Department of Plant Biology, North Carolina State University, Raleigh, NC.
76. A. Jennifer Floyd. Ph.D. 2000, North Carolina State University. Nina Allen, co-advisor. Most recently Assistant Professor, Biology Program, University of Guam, Mangilao, Guam.
77. B. Terri L. Weese. Ph.D. 2004, Brigham Young University. Currently Editor, Australian Plant Name Index (APNI), CSIRO Plant Industry, Canberra, Australia.
78. Nicholas Levsen. Ph.D. 2008, University of Kansas. Currently Postdoctoral Fellow, Institute of Arctic Biology, University of Alaska, Fairbanks, AK.
79. Francisco J. Camacho. Ph.D. 1999, Oregon State University. James M. Trappe co-advisor. Currently homemaker, San Juan Capistrano, CA.
80. John Wheeler. Ph.D. 1998, Oregon State University. Currently Associate Professor, Department of Biology, University of Wisconsin, River Falls
81. Barbara Wilson. Ph.D. 1999, Oregon State University. Currently Partner, *Carex* Working Group LLC [botanical consulting firm], Eugene, OR.
82. John Syring. Ph.D. 2006, Oregon State University. Co-advised by Richard C. Cronn, USDA Forest Service PNW. Currently Assistant Professor, Linfield College, McMinnville, OR.
83. Jason Alexander. Ph.D. 2007, Oregon State University. Currently Herbarium Curator, Utah Valley University, Orem, UT.
84. Ann Willyard. Ph.D. 2007, Oregon State University. Currently Post Doctoral Fellow, Department of Biology, University of South Dakota, Vermillion, SD. Assistant Professor, Department of Biology, Hendrix College, Conway, AR starting August, 2009.
85. Brian Knaus. Ph.D. 2008, Oregon State University. Co-advised by Richard C. Cronn, USDA Forest Service PNW. Currently Postdoctoral Research Geneticist, USDA Forest Service PNW, Corvallis, OR.

Society of America (1979–1980) and also served as vice president (1994–1996) and president (1996–1998) of the American Fern Society. He has been an Associate Editor of the *American Fern Journal* since 1973 and was editor-in-chief of *Systematic Botany* from 1992 through 1995. Thus far, three species of plants new to science have been named in his honor: a Caribbean moss, *Macrocoma gastonyi* Norris & Vitt (1973); a Mexican polystichoid fern *Phanerophlebia gastonyi* Yatskievch (1992), and the uncommon allopolyploid *Pellaea gastonyi* Windham (1993).

In addition to his contributions to scientific research and service to several scientific societies, Jerry Gastony has been a caring and skilled teacher of both undergraduate and graduate students. His Vascular Plants course was widely recognized as one of the best courses in the Department of Biology at Indiana University, and in 2001 he was honored with the Department of Biology Senior Class Award for Teaching Excellence in Biology and Dedication to Undergraduates. He has also been a much loved and respected mentor to a small dynasty of graduate students, several of whom have gone on to become eminent plant systematists in their own right (Fig. 2, Table 1). During his tenure as director of the Evolution, Ecology, and Behavior Graduate Program in the IU Department of Biology from 1991 to 2002, this program developed into one the strongest of its kind in the country. Even after Gerald Gastony's retirement in 2006, he has continued to be a major force in pteridology and to interact with many researchers and students in the field.—MICHAEL S. BARKER, Department of Botany, University of British Columbia, 3529-6270 University Blvd, Vancouver, BC V6T 1Z4, CANADA, and Department of Biology, Indiana University Jordan Hall 142, 1001 E Third St., Bloomington, IN 46405-3700 and GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299

Gels and Genetics: The Historical Impact of Isozymes on Paradigm Shifts in Hypotheses about Fern Evolutionary Biology.—Although it is comforting when new discoveries confirm established hypotheses, it is positively exciting when novel techniques and observations demand rejection of reigning textbook concepts. The history of genetics for homosporous ferns is an exemplar of how technical innovations and discoveries lead to significant modifications of our working models in biology. Homosporous ferns were originally placed in the mysterious group called the "cryptogams" because, unlike their "phanerogamic" cousins, their manner of breeding was hidden from obvious observation and investigation. Once botanists began culturing the gametophytes of ferns, their reproductive biology was revealed, and a method for conducting genetic experiments (crosses and progeny rearing) became available. The earliest studies of fern genetics were those of Lang (1923) and Anderson-Kottö (1931), who demonstrated that most ferns showed simple Mendelian inheritance of traits. In 1950, Irene Manton published her magnum opus, ushering in a new era of genetic and biosystematic research on seed-free plants. Manton's extensive survey demonstrated that most ferns had

extraordinarily high chromosome numbers and that often what appeared to be polymorphic species were actually reticulate complexes of diploid species and their allopolyploid derivatives. This research helped to demonstrate the importance of including genetic aspects of species in understanding their origins and their population dynamics.

In the 1970s, Edward Klekowski (1979) brought a renewed focus to fern genetics by developing logically consistent and compelling correlations and hypotheses about the evolutionary biology of homosporous vascular plants. Klekowski observed that because homosporous ferns had potentially bisexual gametophytes they should be highly inbred, and because these plants have high chromosome numbers, they should be polyploid. Klekowski further hypothesized that this polyploidy could represent an adaptive response that would buffer the homozygotizing effects of consistent inbreeding. Genetic variation stored among the several to many homoeologous genomes contained in polyploids could be released by non-homologous pairing mistakes during meiosis. Indeed, Hickok (e.g., 1978) provided evidence consistent with pairing between homoeologs. Klekowski's hypotheses were intriguing because if accurate they provided a different genetic system and different evolutionary trajectory for homosporous vascular plants.

- Population variation would be reduced and polymorphism constrained.
- Polysomic inheritance would require different algorithms for estimating the population genetic dynamics of homosporous vascular plants.
- If single spores could germinate to become bisexual gametophytes that generated sporophyte offspring, wind dispersal and migration would surmount most geographic barriers and lead to large species ranges.

Although some breeding experiments and chromosomal studies proved to be consistent with Klekowski's hypotheses, central implications of them could not be addressed until enzyme electrophoresis provided a window on molecular genetics. Whereas the hypotheses predicted that ferns should have numerous duplicated genetic loci and be predominantly homozygous, isozymes demonstrated that species with generically basal chromosome numbers were genetically diploid and possessed numerous heterozygous loci (Gastony and Gottlieb, 1982; Haufler and Soltis, 1986). These discoveries required revised hypotheses and forced a revolution in modeling population-level phenomena for ferns.

- Mechanisms promoting outcrossing were explored and verified through coordination of laboratory and field studies (e.g., Haufler and Soltis, 1984).
- Given a new (higher base numbers) starting point, polyploidy levels in homosporous vascular plants actually approximated those of other plant groups (Vida, 1976).
- No longer constrained by lethargic rates of change because of polygenic systems, it was reasonable to posit that diploid ferns could adapt and diversify along with their seed plant descendants (Schneider *et al.*, 2004).
- At the species level, migration via single spores became a specialized rather than a standard capacity for ferns (Haufler, 2002).
- Fern biogeographers were required to consider a new variety of possible outcomes from dispersal and vicariance (e.g., Wolf *et al.*, 2001).

- Within populations, standard diploid-based models of population genetics obtain. Most diploids have random-mating breeding systems with inbreeding restricted to specialist species, those with subterranean gametophytes, and (of course) polyploids (Ranker and Geiger, 2008).

Dismissing ferns as stagnant evolutionary dead-ends ceased to be an option, and with exciting new evidence from DNA and genomic studies, new vistas are opening all the time.

Discovering the paradox that ferns had high chromosome numbers but were genetically diploid necessarily led researchers to ask how this unusual condition could have evolved. One hypothesis was that ferns differed (once again) from other organisms and the lineage started with a larger number of chromosomes (Soltis and Soltis, 1987). A second hypothesis stated that ferns (and other homosporous vascular plants) accumulated chromosomes through cycles of polyploidy events, followed by a return to genetic diploidy through gene silencing (Haufler, 1987). Why ferns retain chromosomes after silencing half their genes remains unclear (and does suggest they differ from other organisms), although it may be related to strong genetic control of bivalent formation (multivalents–that can result in chromosome losses–are rare in ferns having a balanced number of chromosome sets). Experiments and observations aimed at testing these hypotheses (Pichersky *et al.*, 1990; Gastony, 1991; McGrath and Hickok, 1999; Nakazato *et al.*, 2008) have all demonstrated that ferns having chromosome numbers that are basic within genera appear to have experienced ancient polyploidy followed by gene silencing. Support for the polyploidy plus silencing hypothesis is also consistent with new evidence that plant genomes are remarkably volatile and fluid (e.g., Adams and Wendel, 2005).

Resolving these genetic mysteries of vascular cryptogams leads to a whole new set of open questions:

- We know little or nothing about the actual processes involved with gene silencing in ferns. What is the mechanism that results in the paradoxical genetic constitution of the homosporous vascular plants?
- The majority of studies on fern genetics have focused on temperate groups. With most diversity in the tropics, and the origin of temperate groups tied to tropical ancestors, we need to know more about how tropical populations work and whether the conclusions drawn from studies of temperate populations apply to tropical ones.
- We still know surprisingly little about the actual mechanisms that control breeding systems in ferns. More studies that coordinate laboratory analyses of gametophyte biology with surveys of natural populations may help to link mechanisms with observed patterns of genetic variation.
- Although assumptions about trends in species migration have been proposed, the processes that actually result in the founding of new populations remains mysterious. What happens when spores arrive in a new location? What limitations are imposed on species migration by outcrossing breeding systems? Again, coordination of lab and field studies may help to resolve these open questions.
- Perhaps the biggest remaining mystery involves the origin of new species. With demonstrations that fern speciation takes advantage of new habitats opened when angiosperms diversify, it may be possible to study early stages in the cladogenetic process of ferns.

These and other vistas await future generations of scientists interested in understanding the fascinating world of homosporous vascular plants and revealing the cryptic nature of their biology and genetics.—CHRISTOPHER H.

HAUFLER, Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045.

Using Plastid and Nuclear DNA Sequences to Redraw Generic Boundaries and Demystify Species Complexes in Cheilanthoid Ferns.—Cheilanthoid ferns constitute a monophyletic group of 400–500 species within the Pteridaceae (Smith *et al.*, 2006; Schuettpelz and Pryer, 2007; Schuettpelz *et al.*, 2007). They are noteworthy for their ability to colonize xeric and semi-xeric habitats, niches that are rarely exploited by other ferns (Tryon and Tryon, 1979, 1982). Relationships within this lineage are highly problematic, and cheilanthoids have been called "the most contentious group of ferns with respect to a practical and natural generic classification" (Tryon and Tryon, 1982: 248). It is not surprising, then, that molecular phylogenetic analyses to date have revealed that most of the larger cheilanthoid genera are polyphyletic (Gastony and Rollo, 1998; Kirkpatrick, 2007; Prado *et al.*, 2007; Schuettpelz *et al.*, 2007; Zhang *et al.*, 2007; Rothfels *et al.*, 2008). Cheilanthoid ferns have long been a topic of interest for Dr. Gerald Gastony, the honoree of this collection of papers. His contributions run the gamut from studies of chromosome numbers and apomixis in *Bommeria* E. Fourn. (Gastony and Haufler, 1976), through genetic analyses of various species groups (Gastony, 1988; Gastony *et al.*, 1992), to documenting tetrasomic inheritance and gene silencing in polyploids (Gastony, 1990, 1991), and maternal inheritance of plastids in *Pellaea* Link (Gastony and Yatskievych, 1992). His phylogenetic studies of cheilanthoids (Gastony and Rollo, 1995, 1998) were the first to demonstrate that *rbcL* sequences could provide a valuable, independent tool for circumscribing genera in this taxonomically controversial group of ferns.

We are now poised to take the "next step" toward redefining generic boundaries among the cheilanthoids. It is clear that the number of genes and taxa analyzed must be significantly increased if we hope to obtain a robust phylogeny of the group. To this end, we have initiated a large-scale phylogenetic study using DNA sequences derived from three plastid regions (*rbcL*, *atpA*, *trnG-R*). To date, we have sequenced all three plastid regions (representing nearly 4000 base pairs) for 157 species. Maximum likelihood analyses of these data identify seven, well-supported subclades of cheilanthoid ferns (Fig. 3).

Ludens clade.—Previously published analyses (Schuettpelz *et al.*, 2007; Zhang *et al.*, 2007) revealed that *Doryopteris ludens* (Wall. ex Hook.) J. Sm. is not closely related to most taxa traditionally placed in this genus, including the type species, *D. palmata* (Willd.) J. Sm. Whereas *Doryopteris* J. Sm. in the strict sense is strongly supported as a member of the hemionitid clade (Fig. 3), *D. ludens* and its close allies appear to represent a rather isolated lineage within the Pteridaceae. Analyses by Schuettpelz *et al.* (2007) resolved *D. ludens* as sister to all other cheilanthoid ferns while those of Zhang *et al.* (2007) suggested a possible affinity to other pteroid lineages. Though the placement of this species varies depending on taxon sampling, it is clear that it

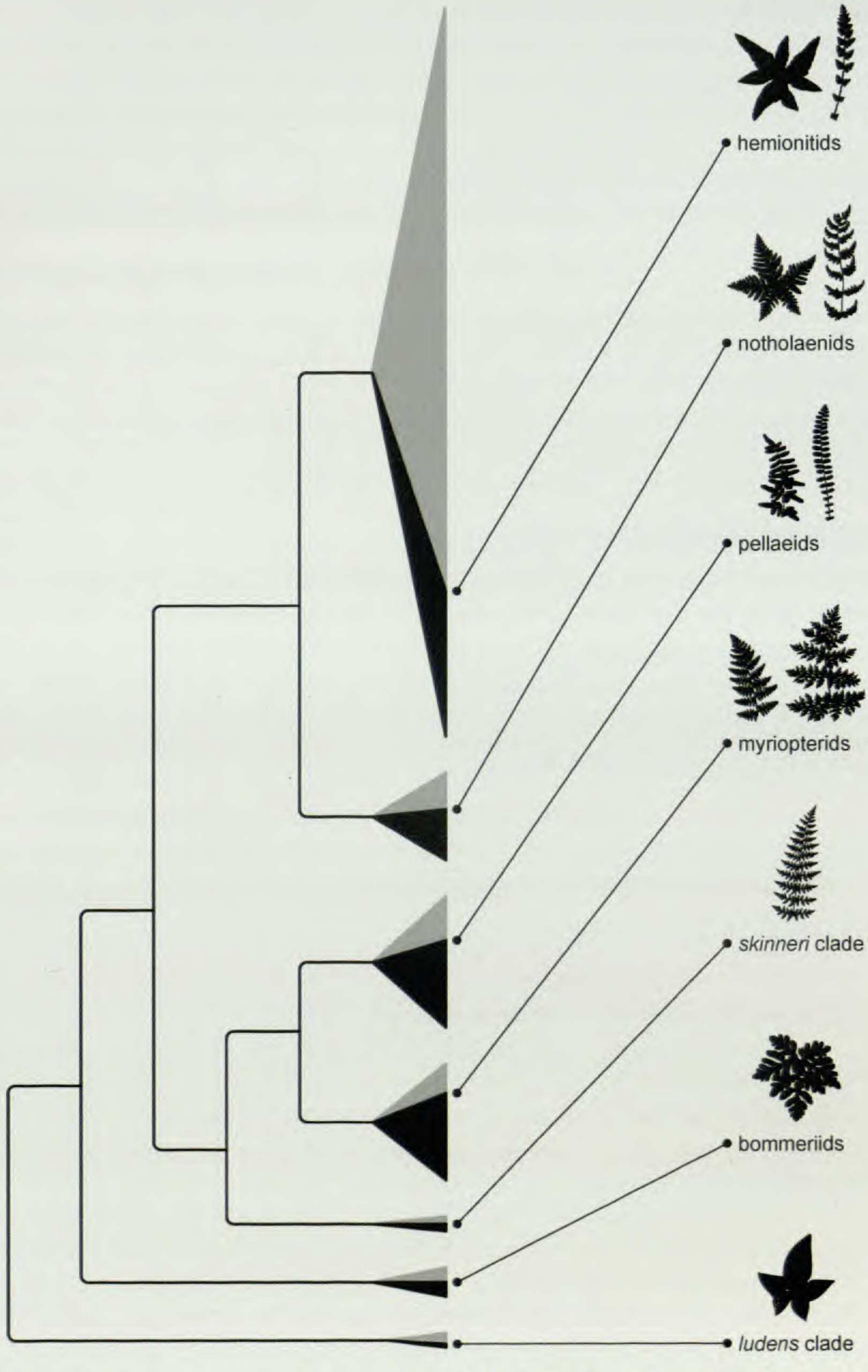

FIG. 3. Summary of phylogenetic relationships within cheilanthoid ferns. Topology results from maximum likelihood analyses of *atpA*, *rbcL*, and *trnG-R* sequence data for 157 species; tree rooted with *Doryopteris ludens*. Thumbnails identify seven, well-supported cheilanthoid clades. Triangles indicate proportion of named species belonging to each clade; darker portion of each triangle represents the proportion of species included in the current analysis.

is more closely related to cheilanthoid ferns than to any other potential outgroup sampled to date. For this reason, we have used it in our analyses to root the remaining cheilanthoid tree. The *D. ludens* clade encompasses a total of four species (only one of which is included in our sample) whose combined range extends from continental Asia to New Guinea. Because its phylogenetic divergence and geographic isolation from *Doryopteris s.s.* are substantial, this lineage is in the process of being transferred to a new genus: "*Calciphilopteris*" (Yesilyurt and Schneider, in press).

Bommeriids.—As shown in earlier studies (Gastony and Rollo, 1995, 1998), species of *Bommeria sensu lato* (including *B. elegans* (Davenp.) Ranker & Haufler; see Ranker and Haufler, 1990) are sister to all cheilanthoids other than the *D. ludens* clade. Our data confirm Ray Cranfill's (unpubl. data) assignment of *Cheilanthes brandegeei* D. C. Eaton to this clade, suggesting that the circumscription of *Bommeria* may need to be expanded yet again. Some species that Tryon and Tryon (1982) considered close relatives of *C. brandegeei* are strongly supported as members of the notholaenid clade in our analyses (Rothfels *et al.*, 2008), and these already have been transferred to *Notholaena* (Yatskievych and Arbelaez, 2008). The remaining members of the "*C. brandegeei* group" (*sensu* Tryon and Tryon, 1982) need to be sampled before the bommeriid clade can be accurately delimited. Based on available data, we estimate that this lineage ultimately will encompass about 2% of cheilanthoid species, half of which have now been included in our analyses.

Skinneri clade.—In a recent parsimony analysis of *rps4*, *rps4-trnS*, and *trnL-F* sequences by Kirkpatrick (2007), *Cheilanthes skinneri* (Hook.) R.M. Tryon & A.F. Tryon was weakly supported as sister to all cheilanthoids other than *Bommeria* (the *ludens* clade was not included in her sampling). In our studies, this taxon is strongly supported as sister to the myriopterid + pellaeid clade; together, these three clades are sister to the notholaenid + hemionitid clade (Fig. 3). Our molecular data also support a close relationship between *C. skinneri* and *C. lozanoi* (Maxon) R.M. Tryon & A.F. Tryon, an association previously proposed based on morphology (Mickel, 1987). Although these species have been transferred back and forth between *Pellaea* (in the pellaeid clade) and *Cheilanthes* (hemionitid clade) in the past, our data indicate that neither generic placement is tenable. Mickel (1987) identified several other taxa that may be related to *C. skinneri*, and these must be sampled before we can adequately circumscribe the clade and determine the correct generic name for it. Based on the available data, we estimate that this primarily North American lineage will include 4–5 species (about 1% of cheilanthoid diversity), two of which were included in the current analysis.

Myriopterids.—This clade encompasses a group of primarily North American species traditionally placed in *Cheilanthes*. A similar assemblage, also sister to the pellaeid clade, was recovered by both Gastony and Rollo (1998) and Kirkpatrick (2007). Our analyses indicate that this group is only distantly related to the type species of *Cheilanthes* (*C. micropteris* Sw., a member of the hemionitid clade) and, as such, all included taxa will need to be transferred to another genus (Grusz *et al.*, in prep.). The type species of *Myriopteris* Fée

(1852), *Cheilosoria* Trev. (1877), and *Pomatophytum* M.E. Jones (1930) all belong to this clade, so there is no shortage of potential names. The challenge will be to identify morphological features that consistently separate this group from *Cheilanthes sensu stricto*. We estimate that this lineage comprises approximately 10% of cheilanthoid diversity; 75% of recognized species have been sampled to date.

Pellaeids.—In addition to *Pellaea s.s.* (described by Link in 1841), this clade includes four genera named within the last 70 years: *Argyrochosma* (J. Sm.) Windham, *Astrolepis* D.M. Benham & Windham, *Paraceterach* Copel., and *Paragymnopteris* K.H. Shing. As revealed by earlier molecular analyses (Gastony and Rollo, 1998; Kirkpatrick, 2007), *Argyrochosma* (with ca. 30 species) is sister to all other pellaeids and can continue to be recognized as a distinct genus as proposed by Windham (1987). The other three genera, although morphologically more divergent than *Argyrochosma*, are nested within the traditional circumscription of *Pellaea* section *Pellaea*. It appears that members of this clade have switched from a typical *Pellaea* morphology (highly divided, nearly glabrous leaves) to an *Astrolepis-Paraceterach-Paragymnopteris* morphology (usually simply pinnate, densely scaly or hairy leaves) on no less than three occasions on three different continents. The taxonomic problems posed by this situation are not easily resolved; Kirkpatrick (2007) provided a good discussion of the potential synapomorphies of each pellaeid subclade and the various nomenclatural options. The pellaeid clade comprises about 12% of cheilanthoid diversity; 65% of the species are represented in our analyses and additional representatives were sampled by Kirkpatrick (2007).

Notholaenids.—This primarily North American lineage, the subject of a recent study by Rothfels *et al.* (2008), is sister to the large, cosmopolitan hemionitid clade. Most of the species included in the notholaenids are farinose, with abaxial leaf surfaces covered by "powdery" (predominantly flavonoid) deposits produced by underlying glandular trichomes. This feature has often been considered a synapomorphy for the genus *Notholaena* R. Br. (*sensu* Yatskievych and Smith, 2003), but our data place two nonfarinose taxa deep within the clade and a strongly glandular, but non-farinose, species as the earliest diverging branch. Additional morphological studies are underway (Rothfels *et al.*, in prep.) to identify characters that can be used to circumscribe an expanded *Notholaena*. This lineage comprises roughly 8% of cheilanthoid diversity; 60% of recognized species have been sampled to date.

Hemionitids.—This is, by far, the largest and most diverse clade of cheilanthoids; its members are found on every continent except Antarctica and the geographic ranges of two species, *Cheilanthes farinosa* (Forssk.) Kaulf. and *C. concolor* (Langsd. & Fisch.) R.M. Tryon & A.F. Tryon, cover most of the subtropics (Tryon and Tryon, 1973). The lineage includes the type species of more than a dozen genera named between 1753 (*Hemionitis* L.) and 1991 (*Pentagramma* Yatsk., Windham & E. Wollenw.). Nearly all of these generic names are associated with well-supported subclades in our analyses, but relationships among these groups are largely unresolved in the plastid tree.

The hemionitid lineage appears to have undergone a rapid radiation (possibly associated with its colonization of new habitats and continents), and much additional data will be needed to clarify generic boundaries in this group. We estimate that this lineage comprises about 67% of cheilanthoid diversity; only 20% of known species are represented in the current analysis.

Future directions.—Ultimately, we hope to include more than 60% of cheilanthoid species in our studies, with a special emphasis on under-sampled diversity hotspots in South America and Africa. The type species of all validly named genera will be sampled, as well as the majority of species of uncertain or disputed relationship. Phylogenetic analyses of these plastid DNA sequences will be used to identify well-supported monophyletic lineages. These clades can then be evaluated for morphological synapomorphies that will provide the foundation for a revised generic classification.—Michael D. Windham, Layne Huiet, Eric Schuettpelz, Amanda L. Grusz, Carl Rothfels, and James Beck, Department of Biology, Duke University, Durham, NC 27708-0339, George Yatskievych, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299, and Kathleen M. Pryer, Department of Biology, Duke University, Durham, NC 27708-0339.

Phylogenetic Use of Inversions in Fern Chloroplast Genomes.—Evolutionary studies at the genome level are nothing new, even in ferns, for which the earliest approaches can be attributed to the cytogenetic investigations of Irene Manton (Manton, 1950). Yet, within a decade of the development of recombinant DNA techniques, researchers were examining genomes through the study of DNA rather than chromosomes. This began with the pioneering work of Jeffrey Palmer and Diana Stein who demonstrated the utility of variation in the chloroplast genome for evolutionary studies in land plants, including ferns (Palmer, 1987; Palmer and Stein, 1982; Stein *et al.*, 1986). Although the chloroplast genome (hereafter plastome) is generally conserved in structure (Palmer and Stein, 1986), it contains sufficient variation to be used at a wide range of phylogenetic scales.

Two general approaches were used to study structural variation in plastomes, both involving restriction site analysis. The first entailed mapping via heterologous probes. This provided data on structural changes which can be informative especially at deep phylogenetic levels (Raubeson and Jansen, 1992). Hasebe (1992) compared the plastome structure of the fern *Adiantum capillus-veneris* to that of tobacco and found that the gene order in *Adiantum* was reversed throughout much of the inverted repeat region. A series of inversions was necessary to explain the difference. Later Stein *et al.* (1992) attempted to examine this aspect of plastome structure across ferns. The study found that *Osmunda* has the tobacco gene order, whereas the remaining taxa studied (a tree fern and several polypods) all had the *Adiantum* gene order, with no additional changes in structure detected. The second approach to comparing plastomes used variation at the sequence level, detected by presence or absence of restriction sites. This approach was used for more

phylogenetically focused studies including polystichoid ferns (Stein *et al.*, 1989), Cyatheaceae (Conant *et al.*, 1994), and the genus *Pellaea* (Gastony *et al.*, 1992). Furthermore, maternal inheritance of the plastome was demonstrated in ferns (Gastony and Yatskievych, 1992).

By the 1990s, DNA sequencing had become feasible for systematists, such that it replaced restriction site analysis as the method of choice. This had several effects. One was that now researchers were more focused on variation in one or a few genes, those for which PCR and sequencing primers were first developed. However, the genome scale approach had been lost. Yet nucleotide variation was so useful that much of the overall framework of fern phylogeny was established (Hasebe *et al.*, 1994, 1995) using the gene *rbcL*, alone at first, but later adding data from additional genes (Pryer *et al.*, 2004).

We posit that evolutionary studies are now moving back to a genome scale perspective. This latest shift is again driven by technological advances, mostly those associated with high-throughput genomics, and the concomitant reduction in cost. Several researchers are starting to examine the highly complex nuclear genomes of ferns, and some of that work was included in this symposium. Our research group is focused on the plastome, of which two complete sequences are available for ferns: *Adiantum* (Wolf *et al.*, 2003) and *Angiopteris* (Roper *et al.*, 2007). Complete genome sequences provide advantages over the earlier mapping approaches: it is much easier to add taxa to a study and there is no need for additional cross probing. Also, the data provide both nucleotide data and genome structure data, deduced from gene order in the genome annotation. Although we do not yet have additional complete fern plastome sequences, we can use the information from *Angiopteris* and *Adiantum* to focus on a few key areas of the plastome. Now that a more robust phylogenetic framework is available for ferns, we can screen appropriate taxa to examine genome reorganization in more detail.

Our research asks two main questions: how phylogenetically informative is gene order, and what are the evolutionary dynamics of genome structure? Gene order can be phylogenetically informative if the individual events that make up a genome reorganization each fall on a different branch of the tree. Alternatively, if there are temporal destabilization events, then a series of rearrangements can occur on the same branch, reducing the number of informative characters, and in some cases preventing the interpretation of actual events (but still providing strong support for one branch). Furthermore, if physical hotspots for rearrangements are common then it is possible that characters of genome structure might be susceptible to homoplasy.

We used the plastome sequences of *Adiantum* and *Angiopteris* to design primers and used PCR and DNA sequencing to determine gene order in representatives of all major lineages of ferns. Here we focus only on a few regions that we know to vary, based on the two complete plastome sequences available. Details of the technique will be published elsewhere. We found that the complex reorganization of the inverted repeat in ferns (Stein *et al.*, 1992) occurred via two main events. *Angiopteris*, *Osmunda*, filmy ferns, and gleichenioid ferns all possess the "tobacco" (ancestral) gene order. The

schizaeoid ferns appear to have undergone one large (approximately 18 kb) inversion. The remaining lineages have a second large inversion which occurred after the first, and the result is the *Adiantum* gene order, with the rRNA genes occurring in the reverse order, as seen in all other land plant lineages studied to date. Thus, this structural reorganization appears to be comprised of two separate events that map consistently onto the fern phylogenetic framework of Pryer *et al.* (2004). However, other smaller rearrangements are composed of several inversions on the same branch, reducing their phylogenetic utility.

Despite major strides in our understanding of fern phylogeny, several key branches remain poorly resolved. One clade that seems to be well-supported is the monilophytes, which include the Ophioglossales/Psilotales, leptosporangiate ferns, marattioid ferns, and the horsetails (Pryer *et al.*, 2001), and we have found a 3 kb inversion that unites this clade. However, resolution among the four constituent lineages remains unclear. Another problematic area is the filmy ferns and gleichenioid ferns, which may be sister taxa, although the support for this is weak (Pryer *et al.*, 2004). As more ferns plastomes are sequenced it should be possible to discover more phylogenetically informative rearrangements that may help address such unresolved issues. Moreover, genome scale data can be used for more than just phylogenetic studies. For example, several plastomes contain nucleotide repeats that may be variable at the population level. Although shifts in the type of data collected may have been driven by advances in techniques, the trend seems to be an increased ability to generate large amounts of data. Thus, future developments will likely depend on the ability to manage and analyze large data sets.—Paul G. Wolf, Aaron M. Duffy, and Jessie M. Roper, Department of Biology, Utah State University, Logan, UT 84322-5305.

Fern Genome Structure and Evolution.—We now know that genome structure is a dynamic entity, and understanding how it evolves is of fundamental importance in biology. Ferns and seed plants are sister groups, and yet they show interesting differences in their genome structure. Hence, comparative analyses of their genome structure provide insights into what is unique in each group and how the genome structure differences evolve.

One major difference between the fern and seed plant genome is their chromosome numbers. Chromosome numbers of ferns, particularly homosporous ferns, are much higher than those of seed plants (Klekowski and Baker 1966), and the underlying cause of this phenomenon has long been of a great interest to biologists. It is traditionally thought that ferns have high chromosome numbers because they are polyploids (Wagner and Wagner, 1980; Grant, 1981). However, Gastony and Gottlieb (1982) showed that, despite their high chromosome numbers, ferns with the lowest chromosome numbers in their genus show isozyme expression patterns typical of diploid organisms.

To resolve the paradox of high chromosome numbers and diploid gene expression in ferns, Haufler (1987) hypothesized that they have acquired their

high chromosome numbers through repeated cycles of polyploidization and genome diploidization via gene silencing. Consistent with the Haufler's hypothesis, Gastony (1991) showed that duplicated genes in a recent tetraploid species have been progressively silenced since the polyploidization event. More recently, Nakazato *et al.* (2006) looked for evidence of past polyploidization event(s) in a 'diploid' fern at the DNA level, by constructing a linkage map of *Ceratopteris richardii* Brongn.. They detected a large number of duplicated genes, one of the highest proportions among past mapping studies in plants, supporting the hypothesis that ferns are polyploids. The distribution of gene duplicates in the genome, however, revealed no apparent homoeologous chromosomes, evidenced by clustering of sets of gene duplicates in different chromosomes. Nonetheless, statistical tests for clustering of gene duplicates at the genome level were highly significant, suggesting that *C. richardii* has a polyploid-like genome structure. Therefore, it appears that *C. richardii* and perhaps other 'diploid' ferns have experienced ancient polyploidization(s), but homeologous chromosomes have been broken up by subsequent gradual chromosomal rearrangements.

Furthermore, mapping the distribution of chromosome numbers on the known fern phylogeny revealed an apparent increase in the base chromosome numbers at the divergence between the water fern lineage and its sister, ca. 200 MYA (Nakazato *et al.*, unpubl.), although many exceptions to the pattern make it premature to draw a firm conclusion. Together with the results from the linkage mapping study (Nakazato *et al.*, 2006) and EST sequence analyses (Barker *et al.*, unpubl.), it can be concluded that ferns probably have experienced ancient polyploidization event(s).

Therefore, results from the past studies have largely support the Haufler hypothesis of repeated cycles of polyploidization and diploidization, and this phenomenon seems to explain the high chromosome numbers in ferns. However, it has become increasingly clear that polyploidization events are ubiquitous not only among ferns, but also among angiosperms (reviewed in Lockton and Gaut, 2005). Therefore, polyploidization events in ferns alone do not seem to explain the higher chromosome numbers in ferns than in seed plants, unless ferns experience more polyploidizations and extinctions of diploids.

Interestingly, the modes of chromosome structural evolution seem to be substantially different between ferns and seed plants, and this may help us to understand why ferns have higher chromosome numbers. Genome size and chromosome number are significantly positively correlated in ferns (Nakazato *et al.*, 2008), which is expected if no significant structural changes occur to chromosomes. However, no such correlation exists in angiosperms or gymnosperms, suggesting that chromosomal structure is highly dynamic in seed plants, but not in ferns. Also, the distributions of genome size and chromosome number are highly skewed toward low values in angiosperms, so there appears to be selection for small genome size and low chromosome number, but not among ferns.

So why are seed plant genomes more dynamic than fern genomes? Although we do not have good answers yet, we can speculate several alternatives. First, because most ferns are homosporous, and seed plants are heterosporous, this difference in reproductive system may induce selection on genome size and chromosome numbers, although the exact nature of this selection is not known. In support of this hypothesis, heterosporous ferns generally have low base chromosome numbers. Alternatively, chromosomal inheritance patterns may be fundamentally different between ferns and seed plants. Although multivalent formation is common among seed plants, in ferns multivalents that may start to form early in meiosis rarely survive to the late prophase stage. Finally, it is possible that ferns and seed plants have some differences in their genome composition. Transposable elements, in particular, are known to have a substantial contribution to genome size, especially in grasses (Bennetzen, 2002). Although highly speculative, it is possible that fern chromosome structure is highly stable because transposable element activity is lower relative to seed plants.

Answers to the question of why ferns and seed plants have different genome structure will come only from detailed empirical studies. It is highly desirable in future studies to investigate what makes up the large fern genomes and how they are different from those of seed plants. Also, we need to conduct hypothesis-driven studies to establish causal links between the genome structure differences and biological differences between ferns and seed plants, such as reproductive systems and chromosomal inheritance.—TAKUYA NAKAZATO, Dept. of Biology, The University of Memphis, 3700 Walker Ave., Memphis, TN 38152

Evolutionary Genomic Analyses of Ferns Reveal that High Chromosome Numbers are a Product of High Retention and Fewer Rounds of Polyploidy Relative to Angiosperms.—Ever since the first chromosome counts of homosporous pteridophytes revealed that they possess astonishingly high numbers of chromosomes, botanists have recognized the unique genomic composition of these plants. Basal chromosome counts for fern genera are significantly higher than similar values from angiosperms (homosporous ferns $n = 57.05$, angiosperms $n = 16$; Klekowski and Baker, 1966), a result that led early workers to assume that as many as 95% of ferns are polyploids. Numerous hypotheses have been proposed throughout the years to explain the origin and maintenance of these chromosome numbers, but Klekowski and Baker's (1966) hypothesis of homoeologous heterozygosity received the most attention as it was supported by early studies.

However, this hypothesis was refuted through a series of convincing isozyme investigations of fern genetics by Gastony and colleagues (Gastony and Gottlieb, 1982, 1985; Haufler and Soltis, 1986; Gastony, 1991). These studies demonstrated that homosporous fern species with the lowest numbers in their genera possess diploid gene expression patterns, and led to a

hypothesis that fern chromosome numbers are the product of numerous rounds of paleopolyploidy.

To test these hypotheses, I analyzed Sanger and 454-sequenced ESTs from four polypod fern species for evidence of ancient genome duplication. My analyses demonstrate that a single genome duplication occurred near the base of the polypod ferns, a lineage that comprises >80% of extant fern diversity. Combined with available fossil data, I also provide the first estimate of fern nuclear genome evolutionary rates with polypodiaceous nuclear genomes evolving at approximately 4.79×10^{-9} subst./syn. site/year and places the ancient genome duplication at 178 +/− 32 MYA.

Assuming that rates of chromosomal loss in ferns are comparable to angiosperms, this is fewer genome duplications than expected, as many angiosperms with much lower chromosome numbers have experienced numerous rounds of genome duplications (Cui *et al.*, 2006). To further elucidate this pattern, I calculated a rate of paleopolyploidzation for angiosperms and ferns from genomic data sets of 192 species (Barker *et al.*, in prep). This rate comparison reveals that, on average, ferns experience approximately half as many paleopolyploidizations as angiosperms.

So, why then do homosporous ferns possess so many more chromosomes than angiosperms? It appears that pteridophyte genomes are simply less dynamic than angiosperm genomes and maintain their chromosomes with higher fidelity. Consistent with this hypothesis of gene silencing with little loss of physical genetic material is the observation of significantly lower gene density in the *Ceratopteris* genome relative to seed plants (Rabinowicz *et al.*, 2005). Additionally, pteridophytes are the only lineage of vascular land plants that have a strong, positive correlation between genome size and chromosome number (Nakazato *et al.*, 2008). Possibly involved in the maintenance of these chromosomes is another peculiar pteridophyte trait, the strong bivalent pairing of chromosomes (Wagner and Wagner, 1980).

Further research is needed to identify the forces and mechanisms driving the striking differences in genome evolution and organization between seed plants and monilophytes. Perhaps the ultimate tool for addressing this question will be whole-genome sequences of homosporous and heterosporous ferns. Considering innovations in sequencing technology and the declining cost of sequencing, we are likely only a few years away from having such data and further elucidating this most outstanding pteridological mystery.—Michael S. Barker, Department of Botany, University of British Columbia, 3529-6270 University Blvd, Vancouver, BC V6T 1Z4, CANADA, and Department of Biology, Indiana University Jordan Hall 142, 1001 E Third St., Bloomington, IN 46405-3700.

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